

## BIOLOGIC TESTS OF TREMOLITE IN HAMSTERS

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Some years ago, we began to test specially prepared samples of minerals for carcinogenicity and fibrogenicity by intrapleural injection into hamsters. In these tests, we found that we could get tumors resembling mesotheliomas by injecting preparations of chrysotile asbestos fibers, provided that we used large enough doses, and provided that we used preparations with large numbers of fibers longer than 20  $\mu\text{m}$  (Smith, 1974; Smith and Hubert, 1974). From experiments with glass fibers, we learned that not only the length, but also the diameter of fibers was important, tumors resulting in hamsters after the injection of fibers 0.75  $\mu\text{m}$  in diameter, but not with fibers 5  $\mu\text{m}$  in diameter (Smith and Hubert, 1974).

Our long-fiber preparations of chrysotile induced extensive, fibrous pleural adhesions, and the occasional tumors came later; whereas our short fiber preparations did not. A question then arose as to whether the tumors were a non-specific result of mesothelial cells becoming trapped in fibrous pleural adhesions where their oxygen supply could be impaired, and malignant change might occur, according to Warburg's theory of carcinogenesis (Warburg, 1956).

**TABLE 1.** Tests of Samples of Tremolite by Intrapleural Injection in Hamsters (Suspension of all samples except 72N were autoclaved before injection.)

Sample Number	Tumors/survivors <sup>1</sup>			Tumors/survivors <sup>1</sup>		
	Dose: 25 mg			Dose: 10 mg		
	350 days	500 days	600 days	350 days	500 days	600 days
14	0/35	0/27	0/20			
275	0/31	0/15	0/3 <sup>2</sup>	0/34	0/14	0/6 <sup>3</sup>
31	2/28	4/9	6/5	1/41	1/19	1/11
72	3/20	5/6	5/1	0/13	1/6	3/2
72N	4/22	9/10	11/2	0/25	0/19	6/9

<sup>1</sup>Numerator = cumulative number of hamsters with tumors related to treatment. Denominator = number survivors

<sup>2</sup>2 additional animals survive

<sup>3</sup>6 additional animals survive

To explore that question we attempted to induce fibrosis by injecting talc, since talc had occasionally been used in surgery in attempts to induce adhesions of pleural surfaces for the treatment of pneumothorax. We bought a commercially available industrial talc, and injected that into the pleural space of hamsters. This material induced very little fibrosis, and no tumors.

The sample of industrial talc that we had used was obtained from a distributor, Whittaker, Clark & Daniels, Inc., in New York City under their label "#13 Talc", which is described by them as a fibrous talc from New York State. It became our sample FD-14, whose physical and chemical characteristics have been previously described, along with results of our biologic tests of it (Smith, 1974). It was found to contain 50% tremolite, 35% talc, 10% antigorite and 5% chlorite.

Since then, we have carried out intrapleural tests in hamsters with 3 different samples of tremolite specially prepared by various milling and separation techniques. Biologic responses to these samples have differed, as shown in Table 1.

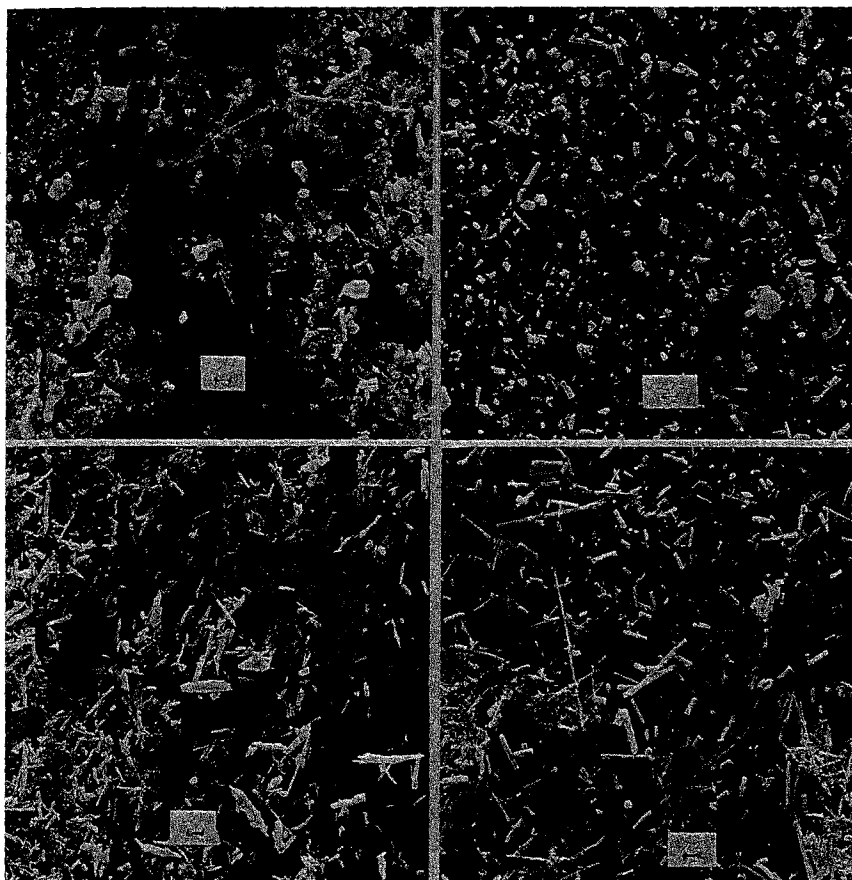
The top line in Table 1 is data from our previously published tests of tremolitic talc (FD-14). It was tested only at our highest dose level (25 mg), and, as shown, no tumors resulted. The other samples (275, 31 and 72) listed in Table 1 are the preparations made from tremolitic ores. Sample 275 was isolated from a sample of tremolite taken from a tremolitic talc ore body similar to those from which FD-14 was produced. Sample 31 was prepared from a sample of tremolite taken from a deposit of tremolitic talc in the western United States. Sample 72 was prepared from a specimen of asbestiform tremolite.

As shown, no tumors related to treatment were found in animals injected with sample 275 at either the 25 mg or 10 mg dose level. A few animals treated with that sample are still living; however, from comparison with the other samples, it appears to be non-carcinogenic. (Animals surviving at time of presentation of this paper were subsequently necropsied, no tumors were found in them).

In contrast, tumors related to treatment were found in some animals injected with samples 31 and 72 at the 25 mg dose level, and less often, at the 10 mg dose level. The first tumor was found 184 days after injection, and most of the tumors by a year or longer after starting the tests. To compare the carcinogenicity of these samples, one must therefore bear in mind the number of animals that survived long into the period of the experiments. The number of survivors at various times, and the cumulative number of animals with tumors related to treatment are shown in Table 1. We conclude that sample 31 is less carcinogenic than sample 72. As shown, at the 10 mg dose, only a single tumor arose in response to sample 31, despite the relative number of animals that survived into late periods of the experiment. Pleural fibrosis was extensive in animals treated with sample 72, less so with 31, and very slight with 275 and FD-14. The fibrogenicity of these samples thus paralleled their carcinogenicity.

For intrapleural injection, our routine procedure has been to suspend mineral samples in saline, and sterilize the suspensions by autoclaving, before injection. In the present work, we injected one group of hamsters with a suspension of sample 72, autoclaved in our usual manner, and we injected another group of hamsters with a suspension of sample 72 that had not been autoclaved. Table 1 shows that more tumors occurred in the group given material that had not been autoclaved, but this may not be significant because the number of survivors in these 2 groups are so different.

When the samples are compared microscopically, morphologic differences can be seen. Figure 1 shows scanning electron micrographs of each sample at x 1250. Comparative measurements of fiber size distributions in those samples are not yet



**FIGURE 1.** Scanning electron micrographs of samples FD-14 (upper left), 275 (upper right), 31 (lower left) and 72 (lower right). Scale on micrographs = 5  $\mu$ m. Original magnification x1250.

available, but differences can be visualized from Figure 1, in which each micrograph bears a 5  $\mu\text{m}$  scale.

The sample that induced most tumors (Number 72) is seen to contain numerous long, thin fibers with parallel sides. Average diameter of these fibers has been calculated as 0.4  $\mu\text{m}$ . By reference to the 5  $\mu\text{m}$  scale on the micrograph, it can be seen that many of these fibers are longer than 20  $\mu\text{m}$ .

The less carcinogenic sample (Number 31) also shows many long, thin particles. Average diameter is 0.5  $\mu\text{m}$ . Some of these particles appear to have parallel sides, but others, although elongated, appear to be rather roughly shaped, resembling acicular fragments rather than crystalline fibers.

Sample 275, which induced no tumors, shows the paucity of long, thin particles so evident in samples 31 and 72. The average diameter of particles in 275 is 0.4  $\mu\text{m}$ . In sample 31, as in 275, some of the elongated particles appear to be fibrous-shaped with parallel sides, but others are rather roughly shaped acicular fragments.

The other sample that proved non-carcinogenic, FD-14, shows long fibers, some thin and some thick, and many platy or amorphous particles. Recall that it contains about 35 % talc. measurements of only the fibrous-shaped particles by optical microscopy at x1000 were earlier reported to show an average diameter of 1.6  $\mu\text{m}$  (Smith, 1974). Measurements of fibrous-shaped particles in the presently available scanning electron micrographs at x1250 show an average diameter of 1  $\mu\text{m}$ .

The negative results with FD-14 may be explained by its lesser content of tremolite, which was 50%, although a relatively low content of tremolite would not explain the negative results with 275. X-ray diffraction studies of 275, 31 and 72 show their tremolite content to be, respectively, about 95%, 90% and 95%.

To what can we attribute the positive results with 72 and 31? Since they contain at least 5% of material other than tremolite, we cannot be sure that their activity is due wholly, or even in part, to tremolite. If we assume that their activity is due to tremolite, then the experiments indicate that appropriately high doses of long, thin particles of tremolite induced tumors, whereas high doses of shorter particles did not. This would, of course, be consistent with previous findings by ourselves and others with other materials, such as chrysotile and glass fibers.

From the point of view of industrial hygiene, it is noteworthy that the experiments show clear-cut, dose-related responses to both preparations that induced tumors. In addition, for estimation of biologic activity of materials containing tremolite, the experiments show that consideration must be given, not merely to the amount of tremolite, but also to other factors, such as the morphologic characteristics of the mineral. Factors of host susceptibility must also be considered. Most tumors in these experiments were diagnosed as mesotheliomas, of which some were examined by electron microscopy and found to contain Type C virus particles (Sobel *et al.*, 1978). Observation of virus particles in the cells of these tumors suggests further work to learn whether a virus is involved in the causation of mesotheliomas.

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